




ORIGINAL RESEARCH

Morphological variation in the common lancehead populations: Sexual dimorphism and ontogenetic patterns

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Keywords

geographic variation; allometry; growth; morphometry; *Bothrops jararaca*; snakes; sexual dimorphism.

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Email: hlucascs@gmail.com

Editor: Anthony Herrel

Associate Editor: Julian Velasco

Received 8 March 2022; revised 16 August 2022; accepted 17 August 2022

doi:10.1111/jzo.13015

Abstract

The common lancehead *Bothrops jararaca* is widespread in the Atlantic Forest in Brazil. The species is known to show a marked sexual dimorphism pattern, with the female being larger than the male. However, most efforts in clarifying morphological variation between the sexes are often focused on a single population. In this paper, we investigate how sexual dimorphism and ontogenetic trajectories vary among populations as well as the ontogenetic trajectories of *B. jararaca*. We analyzed 211 specimens from a coastal and a highland population and measured 17 morphological traits, including linear and meristic characteristics, and the analysis revealed a clear but variable effect of sex and population. Females were larger than males in all evaluated populations. Furthermore, females in the coastal population were generally smaller than in the highland population but had significantly more scales. Widespread species often experience differential environmental pressures even in terms of biotic and abiotic factors. We attribute the results found herein to specificities in prey availability and climatic conditions which affect the ontogenetic pattern between the sexes and the populations, resulting in specific sexual dimorphism patterns.

Introduction

In snakes, sexual dimorphism is a characteristic with ecological impact, being widely shared in the Viperidae family (Hendry et al., 2014). Two hypotheses that possibly explain the differences between the sexes have acquired notable prominence in the last decades. The sexual and fecundity selection hypothesis predicts that being larger carries for one sex certain advantages. In this case, males are larger in species that perform combat behavior and females are larger in species where fecundity rate or offspring number or size are strongly correlated with maternal size (Shine, 1993, 1994). On the other hand, the niche partition hypothesis predicts that morphological differences are due to ecological differences between the sexes such as habitat use or diet (Camilleri & Shine, 1990; Shine, 1986).

The relationship between ecology and morphology in snakes is so complex that significant adaptations may emerge in a short period of time after a drastic change in local dynamics (e.g. introduction of a new species, environmental change). A striking example is a change in body size of Australian snakes associated with the occurrence of the invasive toxic toad *Rhinella marina* (Bufonidae),

where batrachophagous snakes showed a reduction in the size of the mouth opening, limiting the intake of larger and potentially more toxic frogs (Phillips & Shine, 2004).

Widely distributed species generally exhibit morphological variations among different populations. This pattern is mainly associated with differences in the environmental pressures to which each population is subjected. These pressures can be of biotic origin such as eating habits in different types of prey (Aubret et al., 2004; Banci et al., 2022), but also of abiotic origin linked to climate, geography (e.g. altitude, latitude), or phytophysiology (Cruz-Elizalde et al., 2017; Nóbrega et al., 2016; Zhong et al., 2017), which may be a confounding factor when comparing morphology between groups.

Morphometric studies are often focused only on adult individuals; however, the morphological pattern studied may have resulted from early divergence due to ontogenetic development. Ontogenetic allometry hypotheses contrast growth rates in a given variable with body growth, and dimorphism patterns may arise (1) early, if the groups are already born in different sizes and maintain a parallel trajectory, (2) late, if a group grows for a longer time or (3) late, if the groups have different

rates of intrinsic growth (Klingenberg, 1998; for graphical visualization of the patterns above see Sanger et al., 2013).

The common lancehead *Bothrops jararaca* (Wied-Neuwied, 1824) is one of the most emblematic snakes in Brazil, associated with the Atlantic Forest. This forest is located mainly on the coast of Brazil at altitudes between 0 and 1200 a.s.l. Juveniles feed mostly upon anuran amphibians, while adults mostly eat small rodents (Campbell & Lammar, 2004; Marques et al., 2019; Sazima, 1992). Some studies have discovered populations with larger body sizes or marked sexual dimorphism (e.g. Matias et al., 2011; Siqueira & Marques, 2018). Although studies addressing morphological divergences in snakes have been published extensively, many are focused only on one population, sympatric species or address only adults. Therefore, this study aimed to test the hypothesis that populations of *B. jararaca* subject to different environmental conditions may show divergent morphologies. Specifically, we tested the influence of the (1) geographical areas as a source of variation in the direction and degree of intra- and inter-population sexual dimorphism and (2) ontogenetic allometry as a mechanism for morphological divergence.

Materials and methods

We chose to compare populations from two areas with contrasting altitudes and different phytophysiognomies. Individuals from 0 to 80 m a.s.l. were classified as a coastal population whereas those from above 600 m a.s.l. as a highland population, creating a marked gap in elevational gradient which completely segregated the two populations (Fig. 1). The climate in the coastal region is less variable than in the highland and is generally warmer and with higher rainfall over the year (Nimer, 1989; Martinelli, 2009; Fig. S1). The Atlantic Forest formation is predominant in the areas of both populations. However, ombrophilous forest predominates in the coastal region, while the highland includes forest formation, savanna patches, and transitional areas (Martinelli, 2010; Nalon et al., 2020).

We analyzed 211 specimens of *B. jararaca* (Wied-Neuwied 1824): (1) 59 females and 51 males from coastal populations; and (2) 50 females and 51 males from the highland populations, housed in the Herpetological Collection Richard Alphonse Hoge (IBSP), at the Instituto Butantan, São Paulo, Brazil. We measured 17 morphological traits, 15 linear measures, and 2 meristic traits in all individuals. The variables used were as follows: snout-vent length (SVL), number of ventral scales (VS), number of subcaudal scales (SS), tail length (TL), tail width (TW), middle width (MW), head width (HW), the distance between the eyes (DBE), the distance between the loreals (DBL), the distance between the nasals (DBN), distance from eye to nasal (DEN), distance from eye to loreal (DEL), distance from loreal to nasal (DLN), head length (HL), distance from rostral to labial (DRL), head height (HH) and eye diameter (ED; see Fig. 2 for the head schematics and Table 1 for variables descriptions). For the measurements, we used a ruler (precision of 1 mm) or a digital caliper (precision of 0.5 mm). Finally, we analyzed the raw data to calculate the mean and the standard deviation values. For

further analyses, we log-transformed the raw data to achieve normal distributions.

Sexual dimorphism

We considered only adults in the analyses which included females larger than 750 mm SVL and males larger than 650 mm SVL (Sazima, 1992). From the total sample, we included 109 adult specimens, 43 from the coastal population (25F and 18M), and 66 from the highland population (35F and 31M). We analyzed the variation in SVL, VS, and SS between the sexes and the populations using ANOVA with sex, population, and interactions as factors. We further analyzed size-dependent variables using ANCOVA. For TL, MW, and HL, we used SVL as covariate, for TW we used TL, and for all other head measures we used HL as covariable. We run linear models with each dependent variable and its covariate to eliminate the effect of size to identify significant triple interactions. Then, we extracted the residuals of the regression to perform an ANOVA with sex and population as factors with paired Tukey *post hoc* test to access pairwise comparisons.

We computed the Sexual Dimorphism Index (SDI) for SVL as the (mean of female/mean of male)–1 (Shine, 1994). This arbitrary index varies from –1 to 1 and expresses the relative size difference between the sexes, being positive when female-biased, negative when male-biased, and zero when the sexes are equal-sized. Additionally, we used linear discriminant analysis (LDA), which is a multivariate analysis that reduces data dimensions and also predicts individuals into predetermined class. We carried LDA separately for each population to observe the degree of separation or overlap of the sexes combining all morphological variables as a general body shape, as well as which variables have higher discriminating scores between the classes (male or female).

Ontogenetic allometry

We run linear models for each sex and population separately, using 14 variables that co-varied with size. With these models, we aimed to test the hypothesis of the presence of allometry or isometry in each sex and population, and observe the size variation along the individual growth. We then performed an ANCOVA to test the homogeneity of the slope. The presence of a significant result in the interaction is indicative of a difference in the growth trajectory. Similar slopes with intercept statistically significant indicate parallel trajectories, with premature differentiation between the groups. Significantly different slopes indicate divergent trajectories, with late differentiation between the groups. Equal slopes and intercepts indicate no difference in allometric trajectory, and any difference between the groups is simply because one sex may grow more than the other (for graphical representation see Sanger et al., 2013).

Finally, we used a principal component analysis (PCA) to visualize the relationship between the groups in the tangent space. We kept in the analysis only the variables that showed significant results, and we used the residuals of the linear models to avoid bias due to scaling in allometric variables.

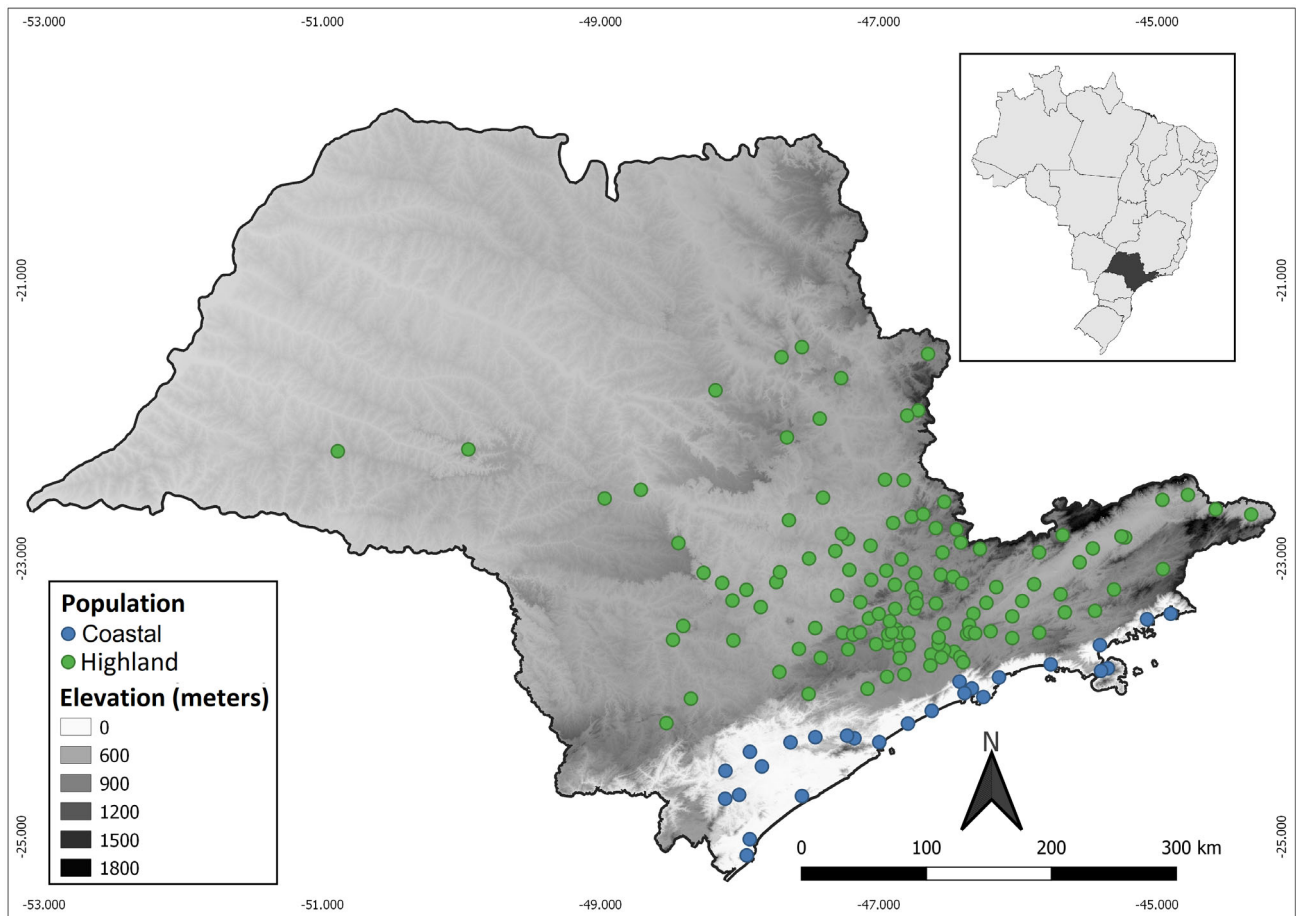


Figure 1 Localities of *Bothrops jararaca* from São Paulo state, Brazil, included in the study.

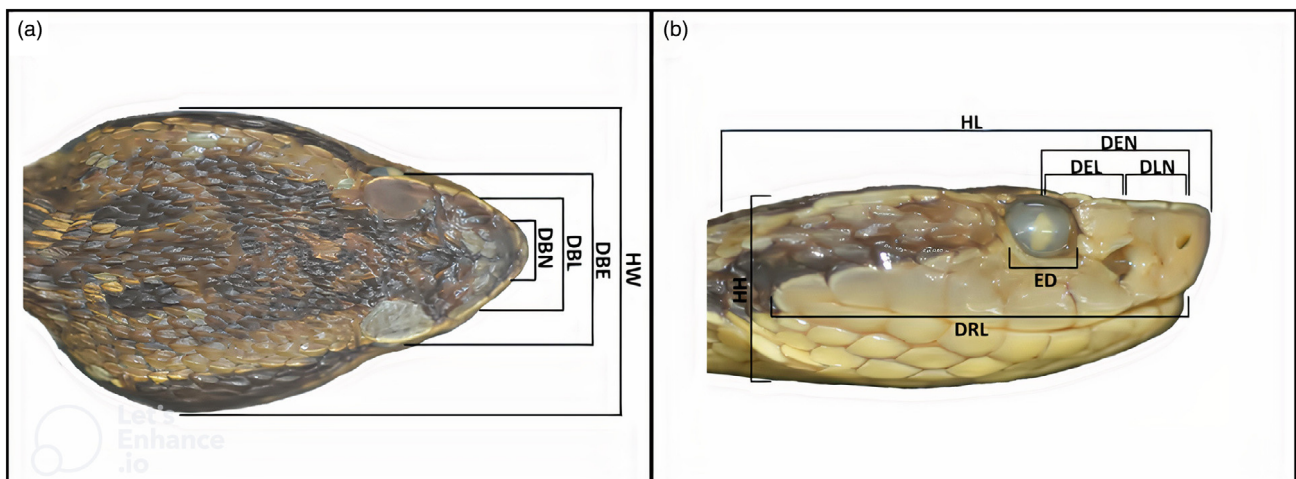


Figure 2 Schematic illustration showing the variables measured in snakes' heads to analyze morphological variation among and within populations of *Bothrops jararaca* in (a) dorsal and (b) lateral view. Head width (HW), distance between the eyes (DBE), distance between the loreals (DBL), distance between the nasals (DBN), distance from eye to nasal (DEN), distance from eye to loreal (DEL), distance from loreal to nasal (DLN), head length (HL), distance from rostral to labial (DRL), head height (HH), and eye diameter (ED).

Table 1 Names and descriptions of the morphological variables used to study *Bothrops jararaca* morphological variation

Variable	Description
SVL	Snout-Vent Length; Measured from the tip of the nose to the anal scale
VS	Ventral Scales; Counted from the first scale post-quadrato bone
SS	Subcaudal Scales; Counted from the first post-anal scale to the tip of the tail
TL	Tail Length; Measured from the cloaca to the tip of the tail
TW	Tail Width; Measured post-cloaca
MW	Middle Width; Total circumference in mid-body
HW	Head Width; Measured in the larger portion of the head (quadrato bone)
DBE	Distance Between Eyes; Measured from right to left subocular scales
DBL	Distance Between Loreals; Measured from right to left loreal pit
DBN	Distance Between Nasals; Measured from right to left nasal scales
DEN	Distance Eye to Nasal; Measured from eye to nasal scales in the right side
DEL	Distance Eye to Loreal; Measured from eye to loreal pit in the right side
DLN	Distance Loreal to Nasal; Measured from loreal pit to nasal scale in the right side
HL	Head Length; Measured from the neck to the tip of the nose
DRL	Distance Rostral to Labial; Measured from the tip of the nose to the last labial scale in the right side
HH	Head height; Measured in parietal region;
ED	Eyes Diameter; Measured horizontally in the middle of the eye

See Fig. 2 for the schematics.

Environmental conditions

To mitigate bias in our conclusions, we directly tested the effect of the environmental variables on the *B. jararaca* phenotype. We obtained annual mean temperature (AMT) and annual precipitation (AP) data from the “Worldclim” database (<https://www.worldclim.org/data/worldclim21.html>; Fick & Hijmans, 2017) at the spatial resolution of 30 arc seconds for each locality of the snakes in both populations. Although there is an altitudinal gap between the two populations, both showed an elevational variation in their distribution, and therefore, we included this variable since the groups were treated separately (see below). To gather elevational data (ELEV), we built a raster with satellite images downloaded from “U.S. Geological Survey” (<https://earthexplorer.usgs.gov/>) and then extracted the elevation data of our data points (Fig. 1). Some error was introduced due to the resolution of our map, and for this reason, two outliers from the coastal population and one from the highland population were excluded from the analysis.

We extracted the vegetation type (VEG) data from the localities based on a raster of vegetation cover downloaded from the “AMBDATA” database (http://www.dpi.inpe.br/Ambdata/mapa_sipam.php) similarly to the elevation data. We assigned individuals into four categories of vegetation types: Atlantic Forest lowlands (ALow), Atlantic Forest submontane (ASub), Atlantic Forest secondary formation (ASec), and Transition from Atlantic to Savanna (TAS). Similarly, an error was introduced as a few locations of the coastal populations fell upon the sea, and in such cases, we considered the vegetation type as the nearest polygon on the map.

To get more robust results, we tested the effects of the environment on morphology using two statistical approaches. First, we used the PC1 scores from the previous PCA as a latent variable and then regressed against each one of the environmental variables using linear models (Jadin et al., 2019). Each sex and population

were treated separately. Secondly, we used a canonical correlation analysis (CCA) to test whether there is an association between the two sets of variables (Manier, 2004). Next, we assessed the statistical significance by calculating *p*-values using “Wilks’ Lambda” *F*-approximation. We also wanted to test each population and sex separately. However, CCA only uses individuals with all the variables available, which resulted in a small sample with high standard deviations for the coastal males, and for this reason, we first tested the coastal females alone and then carried out the test again with both sexes.

Results

Sexual dimorphism

In general, females were larger in their morphology than males, except for the tail variables. Between the populations, the coastal population exhibited smaller morphological values than the highland population, except for the meristic traits (mean and standard deviations given in Table 2). In the same way, the statistical analysis revealed a great variation in morphological patterns, both between the sexes and the populations (Table 3, Fig. 3). Four variables were significantly different only between the sexes (SVL, TL, DEL, and HH), while eight differed only between the populations (VS, SS, TW, MW, DBE, DEN, DRL, and ED). We found a significant effect of the triple interaction for HH and ED. However, those results did not hold after ANOVA was performed on the residuals of linear models and Tukey *post hoc* tests. There was no effect of sex, population, or interactions for the variables HW, DBL, DBN, and DLN.

Females were larger than males for SVL and HL, and had more VS, whereas males were larger for TL and TW, and had more SS. Considering the populations, coastal females had more VS than males and females from the highland

Table 2 Raw data of morphological variables of *Bothrops jararaca* coastal and highland populations

Variable	MEAN \pm SD (mm)			
	F Coastal	M Coastal	F Highland	M Highland
SVL	987.1 \pm 128.9	815.4 \pm 110.3	1038.2 \pm 124.6	800.4 \pm 82.1
VS	204.9 \pm 5.7	199.2 \pm 5.9	196.7 \pm 5.4	191.7 \pm 4.9
SS	59.1 \pm 2.1	63.5 \pm 4.6	56.6 \pm 3.3	62.1 \pm 2.9
TL	146.7 \pm 22.4	129.6 \pm 13.2	147.7 \pm 18.2	129.8 \pm 16
TW	10.3 \pm 2.1	9.4 \pm 1.6	11.8 \pm 2.4	10.6 \pm 1.4
MW	68.9 \pm 37.8	41.7 \pm 26.1	104.6 \pm 24	73.5 \pm 9.9
HW	27 \pm 5.9	21.3 \pm 3.3	30.8 \pm 3.6	22.2 \pm 3.1
DBE	16.1 \pm 2.1	14.1 \pm 1.8	17.7 \pm 1.7	14.1 \pm 1.6
DBL	12.6 \pm 1.9	10.7 \pm 1.6	14.4 \pm 1.6	11.2 \pm 1.3
DBN	7.6 \pm 1.1	6.2 \pm 0.8	8.9 \pm 1.5	7.2 \pm 1
DEN	11 \pm 1.4	9.1 \pm 1.2	11.8 \pm 1.6	9.3 \pm 1.2
DEL	6 \pm 1	5.2 \pm 0.8	7.3 \pm 1.1	5.6 \pm 0.8
DLN	4.8 \pm 0.7	3.9 \pm 0.4	5.3 \pm 1.1	4.1 \pm 0.6
HL	43.1 \pm 5.3	32.8 \pm 3.7	49.4 \pm 5.8	35.8 \pm 4.2
DRL	34.4 \pm 4.7	27.3 \pm 2.9	38.9 \pm 5.4	28.3 \pm 3.4
HH	15.6 \pm 2.9	12.5 \pm 2	18.2 \pm 2.4	13.2 \pm 2
ED	4.9 \pm 0.7	4.4 \pm 0.5	5.2 \pm 0.5	4.4 \pm 0.5

F, females; M, males; sd, standard deviation. Means are in millimeters (mm). See abbreviations in Fig. 2.

population, while coastal males had more SS than males and females from the highland population. Both sexes from the coastal population were smaller for TW, MW, and HL and were larger for DBE, DEN, and DRL.

In the coastal population, SDI on SVL was 0.17, and in the highland population 0.23. In the LDA, all females and males were correctly classified in both populations, and no overlap occurred (Fig. 4). The best discriminant variables for the coastal population were HL (with negative values on the “x” axis) and TL (with positive values on the “x” axis). In the highland population, the best discriminant variables were VS (with positive values on the “x” axis) and SS (with positive values on the “x” axis). Even though there was no overlap, the coastal sexes were closer to each other than the highland population when considering the overall body shape, and together, SDI and LDA strongly indicated a higher disparity between the sexes in the highland population.

Ontogenetic allometry

Allometry hypotheses were rejected once for MW in coastal males ($r^2 = 0.07$, $p = 0.08$); that is, all dependent variables increase with independent size variables increasing, except MW, which remained constant for coastal males (Fig. 5). In general, the percentage of variation explained by the size scaling was quite close in both sexes and populations. Only in two of the 56 models built, the percentage of variation explained by size was below 70% (64% for DLN and 42% for ED in males from the coastal population).

Significant effects in the triple interaction (covariate:sex:population) were not found, indicating homogeneity of slopes when comparing alternated sexes between populations (for example, coastal males with highland females). For the variables HW, DBL, DEL, and DRL, there were no significant

effects of sex, population, or interactions, with equivalent intercepts, and females reached higher values just because they had a longer duration of systemic growth. Nevertheless, a significant effect of double interactions occurred in six variables (covariate:sex or covariate: population), indicating a difference in the inter- or intra-population allometric trajectory. The trajectory for TL was parallel and had different intercepts for sex, with males being the larger one, and the variables TW, DBE, DBN, and ED also had parallel trajectories and different intercepts for the populations, where the coastal snakes showed the highest values, indicating early morphological divergence.

The variable HH showed a significant interaction between the factors sex and population, which points to the parallelism between the trajectories; however with alternation of the larger sex, that is, on the coastal population, males had larger HH than females, whereas in the highland population the opposite occurred. The variables MW, DEN, and DLN showed significant interactions between the covariate and population, indicating non-parallel trajectories. Finally, HL showed significant interactions for the covariate and sex and the covariate and population, indicating different trajectories in these two factors (Fig. 5).

For the PCA, we used the residuals of the linear models for the 10 variables above that presented significant results. The first two axes were responsible for capturing 48.6% of the data variation (Fig. 6). It is possible to observe a clear separation between adult males from the coastal population and adult females from the highland population, while the other groups overlapped in the center. The variables HL and MW had the highest negative values on the PC1 axis, while ED and DEN were higher in the positive direction. This axis relates to the segregation in the distribution among adults, with males from the coastal population having mainly greater eye diameter and

Table 3 ANOVA and ANCOVA results of the morphological variation between the sexes and populations (coastal and highland) of *Bothrops jararaca* (dependent variables and predictors shown)

Dependent	Predictors	F	p
SVL	Sex	99.55	<0.001
	Population	0.88	0.34
	Sex:Population	1.96	0.16
VS	Sex	27.80	<0.001
	Population	54.27	<0.001
	Sex:Population	0.05	0.823
SS	Sex	55.34	<0.001
	Population	9.01	0.003
	Sex:Population	1.06	0.30
TL	SVL	160.62	<0.001
	Sex	6.41	0.01
	Population	0.05	0.82
	SVL:Sex	0.22	0.63
	SVL:Population	0.53	0.46
	Sex:Population	1.70	0.19
	SVL:Sex:Population	1.03	0.31
TW	TL	47.20	<0.001
	Sex	0.72	0.39
	Population	12.09	<0.001
	TL:Sex	0.30	0.58
	TL:Population	0.34	0.55
	Sex:Population	1.08	0.3
	TL:Sex:Population	1.67	0.19
MW	SVL	21.62	<0.001
	Sex	0.36	0.54
	Population	43.00	<0.001
	SVL:Sex	1.26	0.26
	SVL:Population	1.59	0.21
	Sex:Population	0.16	0.68
	SVL:Sex:Population	1.33	0.25
HW	HL	364.95	<0.001
	Sex	0.01	0.90
	Population	0.81	0.36
	HL:Sex	0.34	0.56
	HL:Population	0.30	0.58
	Sex:Population	2.58	0.11
	HL:Sex:Population	0.40	0.52
DBE	HL	387.69	<0.001
	Sex	0.34	0.55
	Population	6.09	0.01
	HL:Sex	0.20	0.64
	HL:Population	0.35	0.55
	Sex:Population	0.90	0.34
	HL:Sex:Population	0.06	0.79
DBL	HL	393.43	<0.001
	Sex	1.19	0.27
	Population	0.62	0.43
	HL:Sex	0.00	0.97
	HL:Population	0.02	0.88
	Sex:Population	0.82	0.36
	HL:Sex:Population	0.22	0.63
DBN	HL	215.46	<0.001
	Sex	3.18	0.07
	Population	3.90	0.05

Table 3 Continued.

Dependent	Predictors	F	p
DEN	HL:Sex	0.03	0.85
	HL:Population	1.04	0.31
	Sex:Population	0.29	0.58
	HL:Sex:Population	0.04	0.83
	HL	301.17	<0.001
	Sex	0.27	0.60
	Population	6.91	0.009
DEL	HL:Sex	2.40	0.12
	HL:Population	0.01	0.92
	Sex:Population	0.02	0.87
	HL:Sex:Population	0.03	0.85
	HL	269.92	<0.001
	Sex	4.52	0.03
	Population	1.42	0.23
DLN	HL:Sex	0.00	0.98
	HL:Population	1.33	0.25
	Sex:Population	0.19	0.65
	HL:Sex:Population	0.00	0.97
	HL	167.84	<0.001
	Sex	0.10	0.74
	Population	1.46	0.230
HL	HL:Sex	0.34	0.5
	HL:Population	0.17	0.68
	Sex:Population	2.53	0.11
	HL:Sex:Population	0.35	0.55
	SVL	634.97	<0.001
	Sex	25.50	<0.001
	Population	45.24	<0.001
DRL	SVL:Sex	0.12	0.72
	SVL:Population	1.08	0.29
	Sex:Population	0.38	0.53
	SVL:Sex:Population	0.78	0.37
	HL	1341.79	<0.001
	Sex	0.96	0.32
	Population	8.09	0.005
HH	HL:Sex	2.36	0.12
	HL:Population	1.57	0.21
	Sex:Population	2.70	0.10
	HL:Sex:Population	0.00	0.94
	HL	429.74	<0.001
	Sex	0.44	0.50
	Population	0.25	0.61
ED	HL:Sex	0.33	0.56
	HL:Population	0.03	0.85
	Sex:Population	4.39	0.03
	HL:Sex:Population	2.69	0.10
	HL	155.19	<0.001
	Sex	0.53	0.46
	Population	5.09	0.02
	HL:Sex	2.64	0.10
	HL:Population	1.44	0.23
	Sex:Population	2.75	0.10
	HL:Sex:Population	4.47	0.03

F, F-test; p, p-value. See Fig. 2 for the abbreviations. Significant differences are in bold.

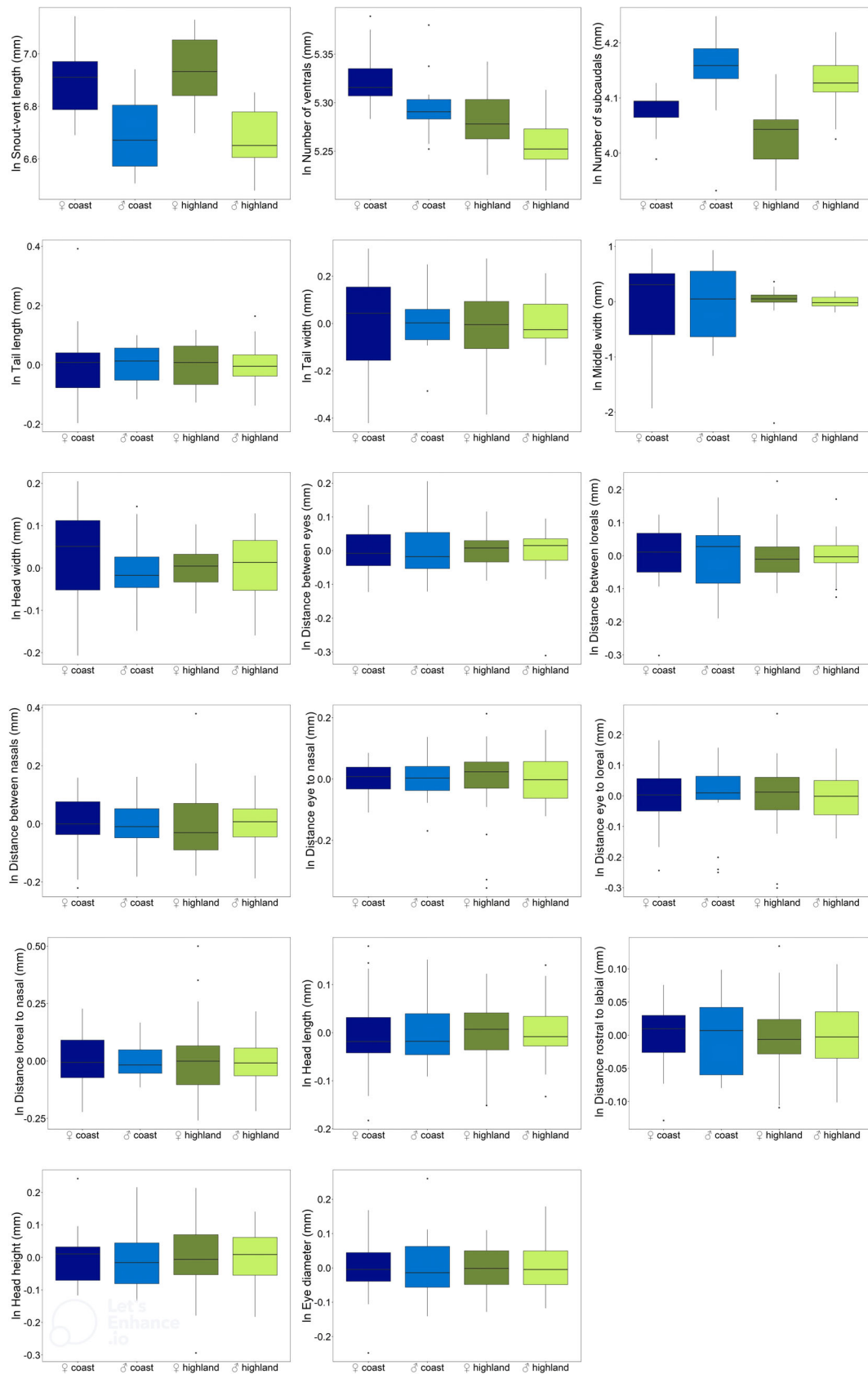


Figure 3 Boxplots showing sexual dimorphism in two populations of *Bothrops jararaca*. The top panels are raw data, and the other plots are residuals extracted from linear models between the target variable and covariable (see Table 3) to exclude the effect of size. All variables were previously log-transformed. The lines are medians; the boxes and whiskers are the quantiles.

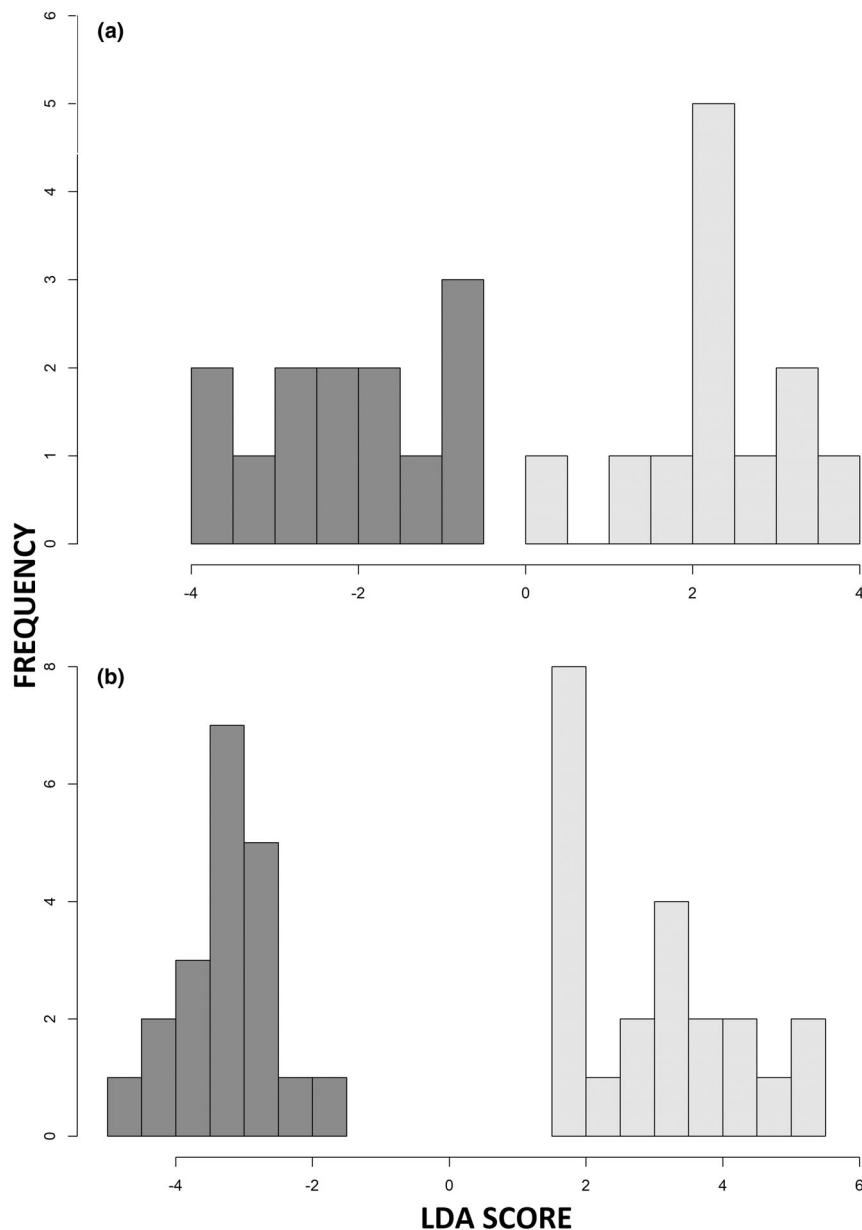


Figure 4 Linear discriminant analysis between the sex categories of *Bothrops jararaca* based on 17 morphological variables. The x-axis represents the discriminating scores for the coastal population (a) and highland population (b). Dark gray bar = females; Light gray bar = males.

greater distance between the eye and the loreal pit, and females from the highland population were more robust and had larger heads. On the PC2 axis, ED and DEN were higher in the negative direction and MW and TL in the positive

direction. This axis relates to the separation between young and adult specimens, with the former having a larger diameter of the eye and the distance between the eye and nostrils. Overall, adults were more robust and had a relatively larger tail.

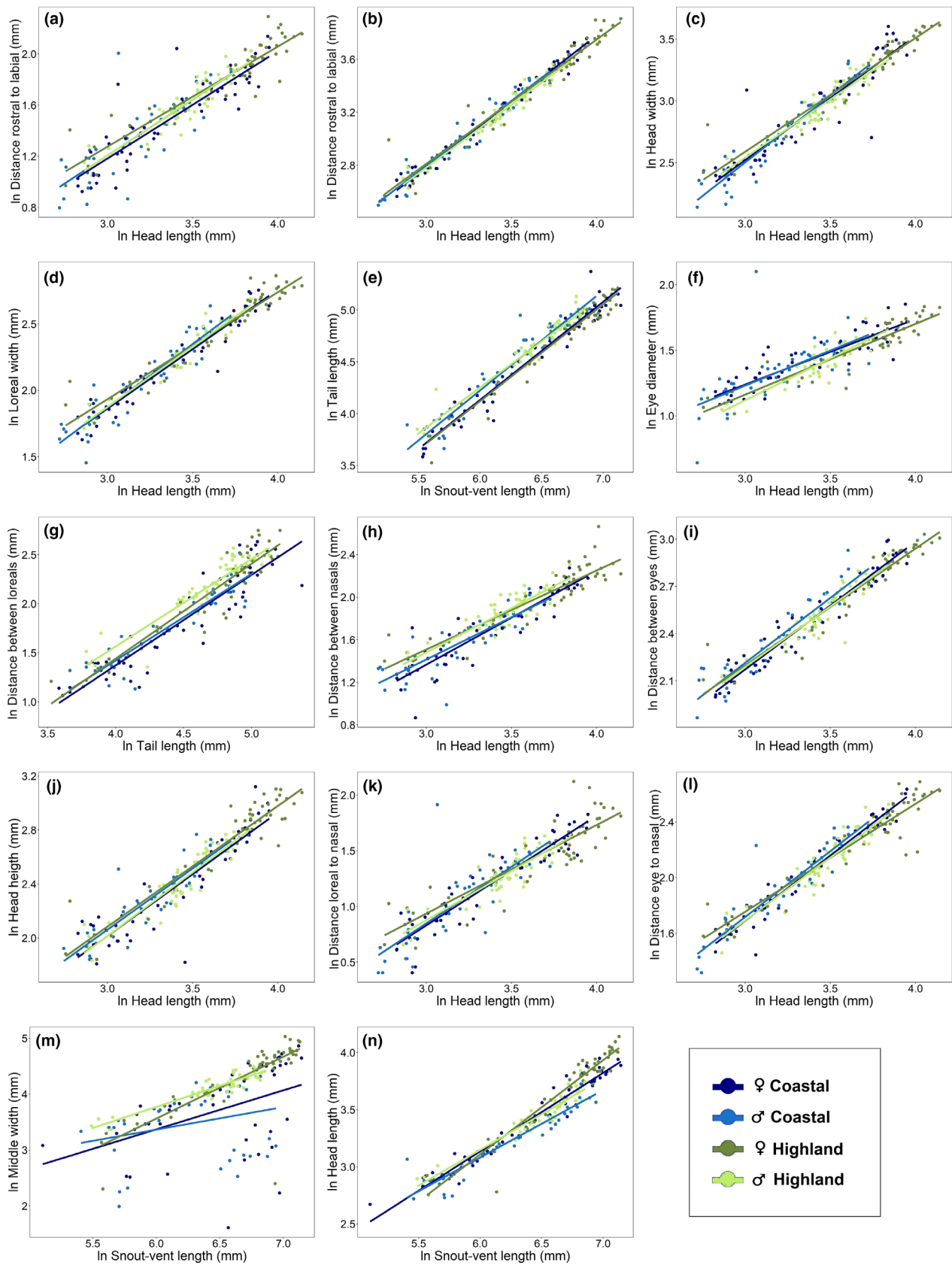


Figure 5 Ontogenetic allometry of morphological traits of female and male *Bothrops jararaca* from the coastal and highland populations. (a-d) Equal intercepts and parallel trajectory, (e-j) different intercepts and parallel trajectory, and (k-n) different intercepts and non-parallel trajectory.

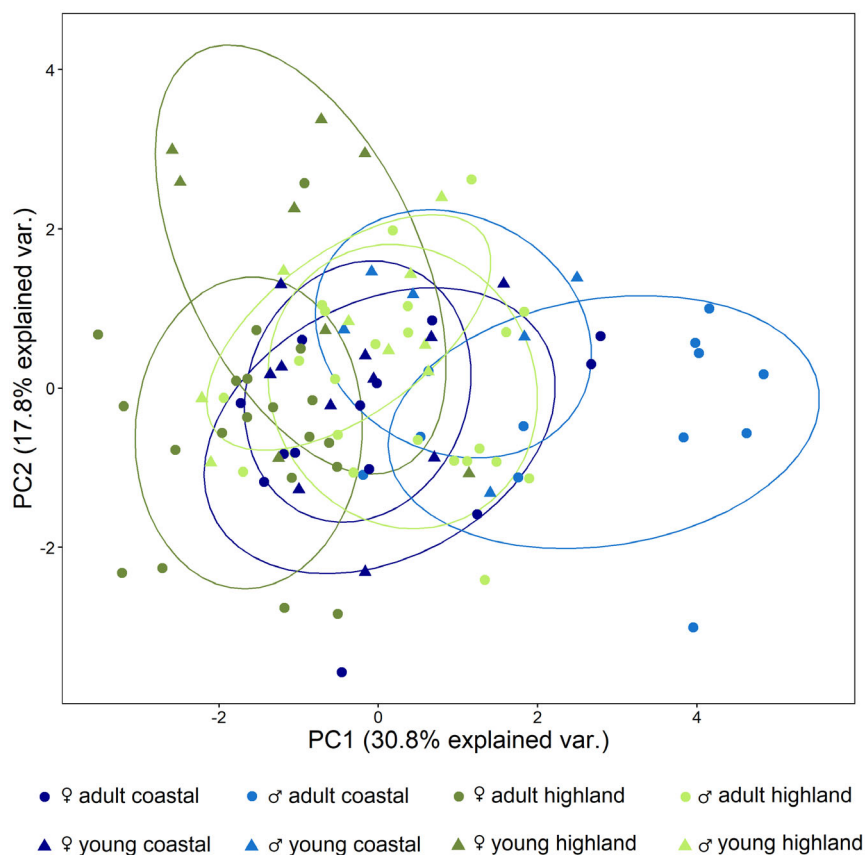


Figure 6 Principal component analysis of the ontogenetic morphological variation between females and males of *Bothrops jararaca* from the coastal and highland populations.

Environmental conditions

All linear models indicated that the four environmental variables did not affect morphology in both sexes and populations ($p > 0.05$; Table S1). Accordingly, any dimensions of the CCA were significantly correlated ($p > 0.05$; Table S2). Combined, the two tests strongly indicated that the environment did not influence the morphology of both populations studied.

Discussion

Sexual dimorphism

Our results indicated considerable morphological differences between and within sexes, but the direction of the variation often alternated between the groups depending on each variable. These results are consistent with other species of viperids (Hoyos et al., 2003; Matias et al., 2011; Sasa, 2002; Zhong et al., 2017), suggesting that the sexes have different

mechanisms of divergence and are influenced by different factors along their distribution. A previous study carried out on *B. jararaca* in southern Brazil showed that several morphological traits differ between the sexes (Matias et al., 2011), with females being almost 20% larger than males. However, the mean size values found in these females were lower than in the females of the highland population and similar to the coastal population. This indicates that morphological characteristics may vary according to different environmental pressures.

The morphological archetype of females with larger body sizes and smaller tails than males is the most common among snakes that lack combat behavior between males (King, 1989; Shine, 1993; Shine, 1994) and is largely consistent with the hypothesis of fecundity selection. Larger females can produce more offspring, which provides great adaptive advantages. Tail characteristics (size, width, and the number of scales) that are greater in males are probably a consequence of the accommodation of copulatory organs, and our finding is a new piece of evidence of the pattern that is extensively found in snakes (King, 1989).

Nevertheless, not all the variations may be explained by being sexually driven, especially considering the trophic morphology (e.g. traits of the head). Females of the carpet python *Morelia spilota* feed on relatively larger prey than males and consequently are also larger, stouter, and have relatively larger heads (Pearson et al., 2002). These ecological divergences, such as diet or habitat partitioning, are generally accompanied by adaptive variations in morphology (Shine, 1989, 1991). Therefore, differences in body size and stoutness of *B. jararaca* could be associated with differences in trophic ecology between the sexes.

Morphological traits may be a result of genetic variation. The number of temporal and ventral scales and color patterns associated with anti-predator behavior in species of *Thamnophis* are known to have a genetic correlation (Brodie III, 1983; Dohm & Garland-Jr, 1993). Although the populations of our study belong to the same phylogroup (e.g. northern clade; Graziotin et al., 2006) and are geographically close, the abrupt altitudinal difference imposed by the Serra do Mar, and consequent physiographic variations, possibly represent a barrier, isolating and partially preventing gene flow.

Hoge et al. (1976) reported a wide variation in the number of *B. jararaca* ventral scales. However, the latitudinal and consequently climatic influence is well marked, as specimens from the southern part of the distribution have considerably smaller numbers of ventral scales in comparison to those from the north. Yet, individuals in the State of São Paulo have an intermediate number of scales, accounting for most of the overlap in data distribution. Accordingly, the most interesting finding in this study is that the populations are very close to each other, and the altitudinal variation is a determinant of variation in the climatic conditions and is probably related to the variation in the number of scales.

A macroecological study found a positive correlation between the scale count and geographical elevation in the *Bothrops* genus (Jadin et al., 2019). However, we found an opposite intraspecific variation which means that the scale count may vary in smaller geographical scales. The number of ventral and subcaudal scales is strongly related to the number of vertebrae, and consequently to the macrohabitat, with the density of vertebrae increasing with arboreal habitat (Banci et al., 2022; Hampton, 2011). Therefore, the largest number of scales in specimens of the coastal population associated with smaller sizes indicates higher scale densities and suggests a most accentuated use of arboreal habitat. Observational and/or experimental studies monitoring specimens in the field may elucidate this issue (cf Banci et al., 2022).

Several traits of the head varied between the populations. The highland snakes have a longer head but the distance between the eyes, distance from eye to the nostril, and distance from the rostral to the last labial scale are greater in the coastal population. In snakes with generalist diet habits, the type of prey consumed may lead to variations in the shape of the head. In *Notechis scutatus* (Elapidae), the population that consumes larger prey also has a larger jaw and mouth (Aubret et al., 2004).

We found no effect of sex or population on the eye size of adults. Although some individuals of *B. jararaca* can be found

actively foraging, this species is known to be an ambush predator (Sazima, 1992) probably relying on other senses such as thermoreception. Experiments on naturally blind snakes or those partially deprived of vision have shown that biological traits and behaviors, such as body condition, prey capturing rate, and finding sexual partners are seldom affected by poor or lack of vision (Bonnet et al., 1999; Young & Morain, 2002), which indicate that the size of the eye per se may not undergo strong natural selection in adults. Additionally, *Bothrops sazimai* (Viperidae), an insular species closely related to *B. jararaca*, have relatively larger eyes than the *B. jararaca* population and are more efficient to detect their ectothermic prey, such as centipedes and lizards (Barbo et al., 2016). In this sense, eye size is probably more variable in juvenile *B. jararaca*, as ectothermic prey are more frequently found in their diet at this stage. Nonetheless, we recommend caution when interpreting this result, since other features of the eye, like cell types and the topography on the retina, are important in hunting and locomotion (Hausman et al., 2014) and may be at least as significant as eye size.

Coastal females and males were less differentiated from each other than females and males in the highland population (e.g. degree of sexual dimorphism). The morphological disparity between the sexes can be more or less accentuated due to the spectrum of the ecological niche occupied by each sex in different populations. In sea snakes, for example, in regions where large prey is less abundant, the degree of sexual dimorphism is often reduced (Shine et al., 2002). This suggests a niche partitioning among the populations of this study.

The linear discriminant analysis shows marked dimorphism between the sexes in both populations. The lack of overlap, however, may have occurred due to the reduced sample, since the sample used contained only individuals with all the variables recorded. Even so, it is possible to see that different variables were responsible for the separation, indicating morphological adaptation in each environment. It is still necessary to keep in mind that many other factors can contribute to sexual segregation, for example demographic variables, and that these may cause bias in the male–female ratio and expression of the SDI, such as parasitism, nutritional stress, or physical exhaustion (Giery & Layman, 2019).

Ontogenetic allometry

Females reached larger sizes (except for the tail attributes) by different mechanisms. Overall, ontogenetic trajectories were parallel between the sexes, and females were larger to begin with, or simply because they attained larger body sizes. Ontogenetic trajectories were also parallel between the populations, and highland individuals were larger since the early stages or because they attained larger body sizes than coastal individuals.

The ontogenetic growth patterns found in this work are very similar to the population in southern Brazil (Matias et al., 2011) and to other species of the genus (e.g. *Bothrops atrox*; Silva et al. 2017). Therefore, this seems to be a highly conserved trait in the genus *Bothrops*. In some traits, females are larger from birth. This appears to be common in snakes, for instance in some matrices where females are larger, most

sexual differences appear soon after birth instead of being fixed by adulthood (Gregory, 2004).

For some traits, even though males were initially equal-sized or even larger, the female growth rate was faster, surpassing males as snout-vent length increased. In snakes, growth rate is rapid initially and decreases after sexual maturity, and in many cases where sexual dimorphism tends towards larger females, they tend to mature later which can result in the observed allometric pattern (Brown & Weatherhead, 1999; Shine, 1978; Webb et al., 2003). Sazima (1992) estimates that females of *B. jararaca* mature at about 750 mm, while males mature at 650 mm.

Skewed survival rate for one sex may culminate in size disparities. Although this factor has not been explored in the present study, the discrepant allometric trajectory between the sexes suggests that the smaller growth rate in males is the most likely factor causing dimorphism, rather than a higher mortality rate. Similar results were found for *Morelia spilota* (Pythonidae) where females showed extreme values for size; however, the recapture rate was equivalent between the sexes (Pearson et al., 2002), which supports the growth-rate hypothesis instead of skewed survival rate.

Since snakes have indeterminate growth, life expectancy may cause morphological differences between populations. Specimens of *Elaphe quadrivirgata* (Colubridae) from the island of Tadanae-Jima, Japan, are considered gigantic compared to those of other populations, live longer, and take twice as long to reach their maximum size, with a constant growth rate (Hasegawa & Mori, 2008). The analysis of the ontogenetic trajectories together with the PCA helps to illustrate the difference between the sexes and the populations throughout development and support the hypothesis that specific ecological pressures act on each population, considering that the sexes in both populations are different earlier in life.

Prey availability often fluctuates according to climatic variation. The growth rate of *Liasis fuscus* (Pythonidae) was higher and more constant when born in years with more food available (Madsen & Shine, 2008). Likewise, prey availability (e.g. anurans) and snake fecundity also co-vary annually, and the positive correlation between maternal size and litter size makes larger females more sensitive to prey fluctuations, as the rate at which fecundity increased with body size was higher when frogs were more abundant (Brown & Shine, 2007). Considering that *B. jararaca* feeds on anurans, at least in juvenile stages (Sazima, 1992), differences in prey availability between the populations may also induce important ecological variations.

Environmental conditions

Despite previous studies that demonstrated correlations between morphology and some environmental variables such as climate and vegetation type (Manier, 2004), rainfall (Tingle & Garland Jr., 2021), and elevation (Tingle & Garland Jr., 2021), we found no evidence that environmental variables used had affected *B. jararaca* morphology. We attribute this to two reasons, first, the geographical scale may not be large enough to represent sufficient environmental variation to promote

morphological disparity. Studies that have found correlation between environment and morphology have generally been carried out in larger scales (e.g. country level; Manier, 2004). Secondly, other variables may exert stronger pressures, such as diet which is known to widely affect body and head size and shape (Aubret et al., 2004; Camilleri & Shine, 1990; Shine, 1991).

Conclusion

In this study, we investigated sexual and populational morphological variation as well as ontogenetic allometry in two *B. jararaca* populations. We found marked sexual variation in several body and head traits and also between populations of the same sex, probably driven by sexual and ecological pressures. Sexual dimorphism degree varied between the populations, which means that intrinsic and extrinsic factors may affect the phenotype in these populations. Ontogenetic trajectory also varies between the sexes and the populations; in that, at least three patterns are recognized, parallel and overlapping, parallel with different intercepts, and non-parallel with different intercepts and slopes. In this sense, size increase from early life to adulthood is different according to each variable and group tested, and those patterns account for the unique sexual and populational morphological variation found.

Acknowledgements

We sincerely extend our sincere thanks to everyone who contributed to the completion of this work. To all friends who provided valuable help in specimen biometrics and reading the manuscript and to Vardir Germano and Felipe Grazziotin (Instituto Butantan) who generously helped with data acquisition and material supply.

Conflict of interest

The authors have no conflict of interest to declare.

Author contributions

Lucas H. C. Siqueira: Conceptualization, data acquirement and analysis, writing and editing the manuscript. Carla Piantoni: Academic contribution, revision, writing, and editing of the manuscript. Otávio A. V. Marques: Academic contribution, revision, writing, and editing the manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Annual climate across *Bothrops jararaca* distribution included in this analysis. The first four graphs are from coastal locations and the last from the highland locations. Data was collected from “Instituto Nacional de Metereologia” (INMET) between 2018 and 2019.

Table S1. Linear models of four environmental variables on two *Bothrops jararaca* populations.

Table S2. Results from canonical correlation analysis of morphological and environmental variables of two *Bothrops jararaca* populations assessed with “Wilks’ Lambda” F-approximation.